TECHNIQUES FOR HANDLING MOSQUITO EGG RAFTS AND RAFT SAMPLES (DIPTERA: CULICIDAE)[1]

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ABSTRACT: We review methods for handling mosquito egg rafts and describe new tools and techniques that permit rapid handling of the large numbers of individual *Culex* spp. egg rafts collected during field studies of oviposition behavior and vector surveillance. We also describe a method of taking egg samples from individual rafts that maintains the species mix and proportional numbers of eggs deposited nightly on an ovisite. The tools and techniques presented greatly decrease the laboratory space required for hatching large numbers of eggs collected for species determination.

Studies of mosquito oviposition behavior and vector surveillance programs commonly utilize data derived from counts of eggs, or egg rafts, collected from ovitraps (Briand 1964, Fay and Eliason 1966, Frank and Lynn 1982, Holck, *et al.* 1988, Jakob and Bevier 1969, Maw and Bracken 1971, Reiter 1986, Smith and Jones 1972, Surgeoner and Helson 1978). Such studies frequently involve thousands of eggs or rafts (e.g., Lowe, *et al.* 1973 [9,956 rafts], Madder, *et al.* 1980 [13,606 rafts], Maw and Bracken 1971 [9,077 rafts], Smith and Jones 1972 [2,332 rafts]). A variety of devices have been used to collect eggs for study: a plastic scoop (Arredondo-Bernal and Reyes-Villaneuva 1989), metal spoon (Beament and Corbet 1981), small sieve (Chadee and Small 1988), small vial (Guptavanij and Barr 1985), spatula made of fine brass screen (Ilitis and Zweig 1962), the corner of a culture plate lid (Reiter 1986), a spoon-type tissue section lifter (Weber and Weber 1985), and a wire loop (Woke 1937). In many cases the collected eggs or rafts must be held in individual containers for hatching so species may be determined using larval characters. Containers used for hatching have been mentioned infrequently: 200 ml plastic cups (Madder, *et al.* 1980), culture dishes (Weber and Weber 1985), and 24-well tissue culture plates (Reiter 1986).

Part of our oviposition research requires that we collect and identify many individual rafts each summer. Only two mosquito species oviposit in our study sites: *Culex pipiens* L. and *C. restuans* Theobald, species distinguishable with certainty using characters of the first instar head capsule (see keys by Dodge 1966 and Reiter 1986). Larval identification

[1] Received October 28, 1992. Accepted November 22, 1993.

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is easiest when larvae are dead. The time required for eggs of these species to hatch and the larvae to die (55-65 h) means that considerable material is in the laboratory at any particular time. To reduce space requirements we use 96-well tissue culture plates for collecting and holding eggs until they hatch and larvae can be identified. The wells are filled ca. two-thirds with aged tap water. The small diameter of individual wells in these plates (6.7mm) required us to develop a tool ("raft spoon") for rapid, accurate collection of individual rafts in the field that could be used by relatively inexperienced personnel to collect rafts directly from field ovisites into the wells (Fig. 1). When required, removal of rafts from the wells is equally easy.

The raft spoon is made from brass rod, 4 mm (5/32") in diameter and 12 cm (4-3/4") in length. Brass rod is available from hobby shops; brazing rod of similar diameter should serve as well. Steps in making the spoon are shown in Fig. 2. The taper is 4 cm (1-9/16") long and is formed by filing from each side until the thin end is ca. 0.5 mm (1/32") thick. Both sides are then polished with emery paper. At this stage the thin end is bent to an approximate 9.5 mm (3/8") radius and two punch marks are made where holes will be bored. Holes are required so rafts center on the tip as they are lifted from the surface, instead of sliding off the side. Bending hardens the brass, so the tip must be annealed before holes can be drilled. This is done by heating it to a dull red, then allowing it to cool. The 1 mm (0.04") holes are made with a #60 drill bit, using the punch marks as starting guides, and the square end is rounded with a file. After the holes are drilled, upper and lower surfaces should be repolished to remove burrs. Wrapping the handles with bright-colored plastic tape helps avoid loss of spoons on the ground and, by increasing their diameter, makes them easier to hold.

In some field studies of *Culex* spp. oviposition, we need to identify the species that have laid rafts on test ovisites each night, but without removing complete rafts from an ovisite. Removing a daily sample of rafts for species identification from those laid the previous night would greatly alter larval abundance and would not indicate the exact number of rafts deposited by each species. Removal of complete rafts could also alter species ratio of the remaining rafts and thus might affect attractancy of the site to gravid females of one species or the other (e.g., Andreadis 1977, Hudson and McLintock 1967, Nakamura 1978). To avoid this problem we remove a sample of 15-25 eggs from each raft for hatching and identification and return the sampled raft to the ovisite. This practice allows us to maintain larval populations in test containers that are proportional, in larval numbers, to nightly oviposition. It has the added benefits of maintaining original species ratios and any attractancy due to presence of eggs or immature stages.

Figure 1. Using the raft spoon to place a raft into a well of a 96-well tissue culture plate.

Figure 2. The three stages in making a raft spoon. Left to right: the taper has been filed; tip has been bent and punched; holes have been bored at punch marks and tip rounded.

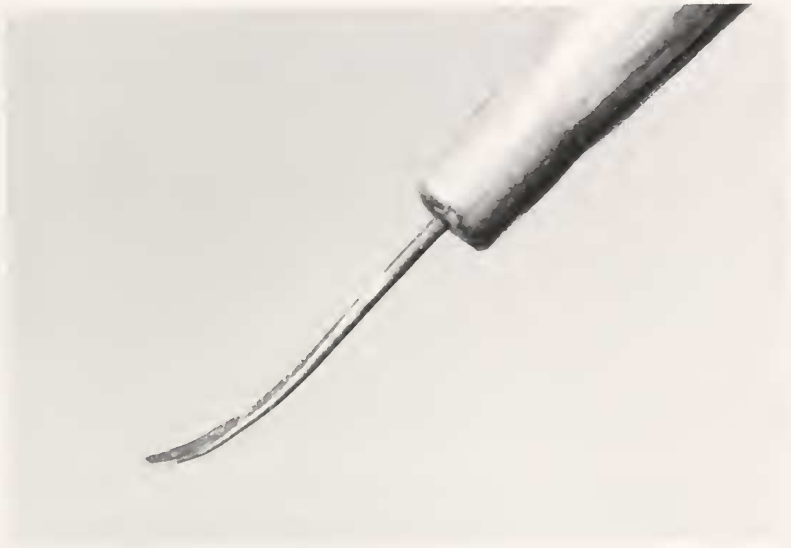

Figure 3. Small spoon for taking samples from egg rafts.

Figure 4. Using the small spoon to obtain a sample of eggs from an egg raft.

To obtain these raft samples, we made a second spoon similar to the one described above, but much smaller and without holes. It consists of a #3 steel insect pin with its tip hammered paper-thin and bent to a radius similar to the first tool. Before bending, the end is rounded with a file and both sides polished with fine emery paper. This tool is set into the end of a wooden handle from an artist's paint brush (Fig. 3).

In practice, a raft is removed from the ovisite surface with the raft spoon, and a sample is removed by slicing off the pointed end of the raft with the edge of the smaller spoon. The smaller spoon is then rotated so it can be slipped under the sample (Fig. 4), and the sample is transferred to a well of a tissue culture plate. The remainder of the raft is placed back on the ovisite, within a floating plastic ring (a fish feeding ring from a pet store) so it will not be sampled again. Eggs hatch within the ring and larvae are free to disperse throughout the ovisite. Both tools are wiped clean after taking a sample to ensure that no loose eggs are carried to the next sample. Data about site and date are written on the culture plate cover with a Sharpie® felt-tip marker (Sanford Permanent Marker Company). Sharpie® markings are easily removed with 95% ethanol when plates are cleaned. We examine each well after returning plates to the laboratory to be sure all samples are upright, which helps ensure hatching (Horner and Weber 1991). It is also necessary to rest one end of the cover on the upper surface of the plate itself, with the other end resting on the table so the two don't seal together from condensation and smother developing embryos (Reiter 1986). For larval identification, the entire culture plate (minus lid) is placed on the stage of a binocular microscope and moved cell-by-cell under the lens.

ACKNOWLEDGMENTS

We thank C. Tipping and S. Kleiner for assistance in raft collection and larval identification. This research was supported in part by Hatch Funds. Published with the approval of the director of the Delaware Agricultural Experiment Station as Paper No. 1484. Contribution No. 653 of the Department of Entomology and Applied Ecology, University of Delaware, Newark, Delaware.

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